IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Yong Soo Chong
Timothy Tully, et al.	Art Unit:1617
Application Serial No.: 09/927,914	Confirmation No: 5180
Filed: August 10, 2001	Attorney's Docket No.43373-0008
For: **Augmented Cognitive Training**	**Customer No. 25213**

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF TIMOTHY TULLY, Ph.D UNDER 37 C.F.R. § 1.132

I, Timothy Tully, Ph.D. declare and say as follows:

1. I was a Professor at Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 from September 1, 1991 until May 31, 2007. I am Chief Science Officer of Dart Neuroscience LLC as of June 1, 2007.

2. I have been Acting Chief Scientific Officer at Helicon Therapeutics, Inc. Farmingdale, NY 11735 since July 1, 1997. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

3. I have been involved in supervising and analyzing the effect of phosphodiesterase inhibitors and training on performance gain during treatment for a cognitive deficit as set forth in the above referenced patent application.

4. I am aware that some of the claims in the above captioned patent application have been rejected under 35 U.S.C. § 112 as allegedly lacking enablement. My understanding is that

the rejection is based, at least partially, on the assertion that it is unpredictable as to whether administration of any phosphodiesterase inhibitor would result in performance gain during treatment of a cognitive deficit associated with a central nervous system disorder.

5. To the contrary, however, it is my considered scientific opinion that administration of any augmenting agent which enhances CREB pathway function by inhibiting a phosphodiesterase in combination with cognitive training would result in performance gain during treatment of a cognitive deficit associated with central nervous system disorder.

6. In support of the conclusion made in paragraph 5 above, I offer the following evidence attached as Exhibit B. In this experiment, the efficacy of the phosphodieterase inhibitor HT0712 in promoting rehabilitation dependent motor recovery and enhancing functional restoration within the motor cortex following cortical ischemia was examined.

7. The experiment described in Exhibit B shows that recovery which is associated with a reinstatement and reorganization of function within residual tissue, can be upregulated via the inhibition of phosphodiesterase in combination with rehabilitative training. With the exception of animals receiving 0.30 mg/kg, all animals receiving the phosphodiesterase inhibitor HT0712 in combination with motor rehabilitation had significantly larger motor maps and better post-stroke reaching performance than vehicle injected controls. The results of the experiment indicate that the phosphodiesterase inhibitor HT0712 contributes to recovery of motor function by augmenting the restoration of cortical function that occurs during rehabilitation.

8. Furthermore, the performance gain during treatment of a cognitive deficit associated with central nervous system disorder can be achieved with any augmenting agent which enhances CREB pathway function by inhibiting a phosphodieterase. Although there are various augmenting agents which may enhance CREB pathway function by inhibiting a phosphodiesterase by a number of different mechanisms, signaling through phosphodiesterase is inhibited, regardless of the manner of inhibition. Thus, the CREB pathway function will be enhanced. Accordingly, ultimately the common mechanism of action of all phosphodiesterase inhibitors is inhibiting the ability of phosphodiesterases to inhibit the CREB pathway function.

9. It necessarily follows from paragraphs 6, 7 and 8 that administration of any augmenting agent which enhances CREB function by inhibiting a phosphodiesterase would result in performance gain during treatment of a cognitive deficit associated with central nervous system disorder.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Signed: _____

Timothy Tully, Ph.D.

Date: 7/30/07

7/30/07 11:16 AM ()

EXHIBIT A

CURRICULUM VITAE **TIM TULLY**

Chief Science Officer	2007	Dart Neuroscience, LLC
Division Head, NeuroGenetics	2006	Cold Spring Harbor Laboratory
Adjunct Professor	2004	Institute of Brain Research National Tsing Hua University, Taiwan
St. Giles Foundation Professor of Neuroscience	2003	Cold Spring Harbor Laboratory
Guest Professor	2002	Department of Biological Sciences Tsinghua University, China
Visiting Scholar	2002	Department of Neurobiology, SUNY Stony Brook
Professor	1995	Cold Spring Harbor Laboratory
Affiliate Professor	1994	Genetics Program, SUNY Stony Brook
Affiliate Professor	1994	Neuroscience Program Cornell University Medical College
Visiting Scholar	1994	Biology Department, New York University
Associate Professor	1991	Cold Spring Harbor Laboratory
Assistant Professor	1987	Biology Department, Brandeis University
Research Associate	1985	Molecular Genetics Massachusetts Institute of Technology
Postdoctoral Fellow	1981	Neurogenetics, Princeton University
Doctor of Philosophy	1981	Genetics, University of Illinois
Bachelor(s) of Science	1976	Biology & Psychology, University of Illinois

Honors, Awards and Professional Activities

Awards Committee, International Behavioral & Neural Genetics Society, 2003 -
Scientific Advisory Board, Joekai Biotech Co., Ltd, 2003 -
Scientific Review Board, Institute for the Study of Aging, 2001 -
Editorial Advisory Board, *Genes, Brain and Behavior*, 2001 -
Acting Chief Scientific Officer, Helicon Therapeutics, Inc., 2001 -
Selection Committee, Lindsley Prize, Society for Neuroscience, 2000 - 2005
Decade of the Brain Award, American Academy of Neurology, 1999
Board of Trustees, The Swartz Foundation, 1997 - 2000
Board of Directors, Helicon Therapeutics, Inc., 1997-
John A. Hartford Foundation Grantee, Cold Spring Harbor Laboratory, 1997-2000
Editorial Advisory Board, *Learning & Memory*, 1995 -
Editorial Advisory Board, *Behavior Genetics*, 1992 -
John Merck Scholarship in the Biology of Developmental Disabilities in Children, 1990 - 1994
Editorial Advisory Board, *Behavioral Neuroscience*, 1989 - 2001
Associate Editor, *Behavior Genetics*, 1987 - 1992
McKnight Scholars Award in Neuroscience, Brandeis University, 1987 - 1990
NIH Postdoctoral Fellow, Princeton University, 1981 - 1985
NIMH Predoctoral Trainee, University of Illinois, 1978 - 1981

Membership in Professional Societies

Society for Neuroscience
Genetics Society of America
American Psychological Association
International Society for Neuroethology
International Behavioural and Neural Genetics Society

Publications (Citations)

Matsuno M., Tully T. and M. Saitoe (2007) The *Drosophila* cell-adhesion molecule Klingon is required for long-term memory and is regulated by *Notch*. (submitted).

Peters M., Bletsch M., Vicdorchic A., Catapano R., Zhang X., Tully T. and & R. Bourtchouladze (2007) RNA interference in hippocampus demonstrates the role of CREB and PP1a in contextual and temporal long-term memory. (submitted).

Wu C.-L., Xia S., Fu T.F., Wang H., Cheng B., Leong D., Chiang A.-S. and T. Tully (2007) NMDA receptors outside of mushroom body are required for long-term memory formation in *Drosophila.* (submitted).

Chen G., Zhang Q.-S., Regulski M., Sinha N., Dubnau J., Tully T., Krainer A.R. and M.Q. Zhang (2007) Characterization of the Nanos-response-element and a search for new *pumilio* targets in *Drosophila* synaptic genes. (submitted).

Xia S. and T. Tully (2007) Different G proteins distinguish odor identity from intensity in *Drosophila* mushroom body. *Public Library of Science Biology* (in press).

Lu Y-B., Lu Y-S., Shuai Y., Feng C., Tully T., Xie Z., Zhong T. and H.-M. Zhou (2007) The AKAP Yu is required for olfactory long-term memory in *Drosophila. Proceedings of the National Academy of Science, USA* (in press).

Keuling A., Yang F., Hanna S., Tully T., Burnham A., Locke J. and H.E. McDermid (2007) Mutation analysis of Drosophila *dikar/CG32394*, homologue of the chromatin remodeling gene *CECR2. Genome* (in press).

Asztalos Z., Baba K., Yamamoto D. and T. Tully (2007) The *fickle* mutation in a cytoplasmic tyrosine kinase effects sensitization but not dishabituation in *Drosophila melanogaster. Journal of Neurogenetics 21*: 59-71.

Qian M., Pan G., Sun L., Liu X., Li F., Tully T., Feng C. and Y. Zhong (2007) Receptor-like tyrosine phosphatase PTP10D is required for long-term memory in *Drosophila. Journal of Neuroscience 27*: 4396-4402.

Joiner M.A., Asztalos Z., Jones C., Tully T. and C.-F. Wu (2007) Effects of mutant *Drosophila* K^+ channel subunits on hatituation of the olfactory jump response. *Journal of Neurogenetics 21*: 45-58.

Asztalos Z., Arora N. and T. Tully (2007) Olfactory Jump Reflex Habituation in *Drosophila. Journal of Neurogenetics 21*: 1-18.

Olson A.J., Tully, T. and R. Sachidanandam (2005) GeneSeer: A sage for gene names and genomic resources. *BMC Genomics 6*:134.

Sharma P., Asztalos Z., Keane J., Silva E., O'Kane C.J., Ayyub C., Madhavan M., Sherkhane P.D., Siddiqi K., Krishnan K., Rodrigues V., DeBruyne M., Carlson J., Dornan A.J., Goodwin S.F., Gomez-Hernandez A., Riesgo-Escovar J., Killeen J., Partridge L., Krammer S., Heisenberg M., Roe H., Kyriacou C.P. and T. Tully (2005) Isogenic autosomes to be applied in optimal screening for novel mutants with viable phenotypes in *Drosophila melanogaster. Journal of Neurogenetics 19*: 57-85.

Xia S., Miyashita T., Fu T.-F., Lin W.-Y., Wu C.-L., Pyzocha L., Lin I.-R., Saitoe M., Tully T. and A.-S. Chiang (2005) NMDA receptors mediate olfactory learning and memory in *Drosophila. Current Biology 15*: 603-615.

Regulski M., Stasiv Y., Tully T. and G. Enikolopov (2004) Essential function of nitric oxide synthase in *Drosophila. Current Biology 14*: R881-882.

Stasiv, Y., Kuzin, B., Regulski, M., Tully, T. and G. Enikolopov (2004) Regulation of multimers via truncated isoforms: a novel mechanism to control nitric oxide signaling. *Genes&Development 18*: 1-12.

Ge X., Hannan F., Xie Z., Feng C., Tully T., Zhou H., Xie Z. and Y. Zhong (2004) Notch signaling in *Drosophila* long-term memory formation. *Proceedings of the National Academy of Sciences, USA 101*: 10172-10176.

Publications (cont.)

Chiang A.-S., Blum A., Barditch J., Chen Y.-H., Chiu S.-L., Regulski M., Armstrong J.D., Tully T. and J. Dubnau (2004) *radish* encodes a phospholipase-A2 and defines a neural circuit involved in anesthesia resistant memory. *Current Biology 14*: 1-20.

Tamura T., Ito N., Chiang A.-S., Tully T. and M. Saitoe (2003) Aging specifically impairs *amnesiac* –dependent memory in *Drosophila*. *Neuron 40*: 1003-1011. **(1)**

Wang Y., Chiang A.-S., Xia S., Kitamoto T., Tully T. and Y. Zhong (2003) Blockade of neurotransmission in *Drosophila* mushroom body impairs odor attraction but not repulsion. *Current Biology 13*: 1900-1904. **(2)**

Broderick K.E., MacPherson M.R., Regulski M.R., Tully T., Dow J.A.T. and S. Davies (2003) Interactions between epithlial nitric oxide signalling and phosphodiesterase activity in *Drosophila*. *American Journal of Physiology – Cellular Physiology 285*: C1207-1218. **(1)**

Broughton S.J., Tully T. and R.J. Greenspan (2003) Conditioning deficits of CaM-kinasè transgenic *Drosophila melanogaster* in a new excitatory courtship assay. *Journal of Neurogenetics 17*: 91-102. **(1)**

Bourtchouladze R., Lidge R., Catapano R., Stanley J., Gossweiler S., Romashko D., Scott R. and T. Tully (2003) A mouse model of Rubinstein Taybi Syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. *Proceedings of the National Academy of Science U.S.A. 100*: 10518-10522. **(5)**

Sengupta R., Sahoo R., Mukherjee S., Regulski M., Tully T., Stuehr D.J. and S. Ghosh (2003) Characterization of *Drosophila* nitric oxide synthase: a biochemical study. *Biochemical and Biophysical Research Communications 306*: 590-597.

Dubnau J., Chiang A.-S., Grady L., Barditch J., Gossweiler S., McNeil J., Smith P., Buldoc F., Scott R., Certa U., Broger C. and T. Tully (2003) The *staufen/pumilio* pathway is involved in *Drosophila* long-term memory. *Current Biology 13*: 286-296. **(18)**

Scott R., Bourtchuladze R., Gossweiler S., Dubnau J. and T. Tully (2002) CREB and the discovery of cognitive enhancers. *Journal of Molecular Neuroscience 19*: 171-177. **(3)**

Pendleton R.G., Rasheed A., Sardina T., Tully T. and R. Hillman (2002) Effects of tyrosine hydroxylase mutants on locomotor activity in *Drosophila*: a study in functional genomics. *Behavior Genetics 32*: 89-94. **(1)**

Stasiv Y., Regulski M., Kuzin B., Tully T. and G. Enikolopov (2001) The *Drosophila* nitric-oxide synthase (*dNOS*) gene encodes a family of proteins that can modulate NOS activity by acting as dominant negative regulators. *Journal of Biological Chemistry 276*: 42241-42251. **(4)**

Dubnau J., Grady L., Kitamoto T. & T. Tully (2001) Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory. *Nature 411*: 476-480. **(69)**

DeZazzo J., Sandstrom D., deBelle S., Velinzon K., Smith P., Grady L., DelVecchio M., Ramaswami M. & T. Tully (2000) *nalyot*, a mutation of the *Drosophila* myb-related *Adf1* transcription factor, disrupts synapse formation and olfactory memory. *Neuron 27*: 145-158 **(15)**.

Kuzin B., Regulski M., Scheinker V., Tully T. & G. Enikolopov (2000) Nitric oxide interacts with the retinoblastoma pathway to control eye development in Drosophila. *Current Biology 10*: 459-462. **(15)**

DeZazzo J.D., Xia S., Christensen J., Velinzon K. and T. Tully (1999) Developmental expression of an amn^{+} transgene rescues the mutant memory defect of *amnesiac* adults. *Journal of Neuroscience 19*: 8740-8746. **(28)**

Pinto S., Quintana D.G., Smith P., Mihalek R.M., Hou Z.-H., Boynton S., Jones C.J., Hendricks M., Velinzon K., Wohlschlegel J.A., Autsin R.J., Lane W.S., Dutta A., and T. Tully (1999) *latheo* encodes a subunit of the Origin Recognition Complex and disrupts neuronal proliferation and adult olfactory memory when mutant. *Neuron 23*: 45-54. **(41)**

Publications (cont.)

Rohrbough J., Pinto S., Mihalek R.M., Tully T. & K. Broadie (1999) *latheo*, a Drosophila gene involved in learning, regulates functional synaptic plasticity. *Neuron 23*: 55-70. **(30)**

Moore M.S., DeZazzo J., Luk A.Y., Tully T., Singh C.M. & U. Heberlein (1998) Ethanol intoxication in Drosophila: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell 93*: 997-1007. **(114)**

Connolly J.B. & T. Tully (1998) Behaviour, learning and memory. In D.B. Roberts (Ed.) *Drosophila a practical approach, Second Edition*. Oxford University Press (Oxford), pp. 265-318.

Goodwin S.F., Del Vecchio M., Hogel C., Tully T. & K. Kaiser (1997) Defective learning in mutants of the Drosophila gene for a regulatory subunit of cAMP-dependent protein kinase. *Journal of Neuroscience 17*: 8817-8827. **(33)**

Mihalek R., Jones C. & T. Tully (1997) The *Drosophila* mutation *turnip* has pleiotropic behavioral effects and does not specifically affect learning. *Learning & Memory 3*: 425-444.

Connolly J.B., Roberts I.J.H., Kaiser K., Forte M., Tully T. & C.J. O'Kane (1996) Associative learning is mediated by G_s signaling in *Drosophila* mushroom bodies. *Science 274*: 2104-2106. **(128)**

Li W., Tully T. & D. Kalderon (1995) Effects of a conditional *Drosophila* PKA mutant on learning and memory. *Learning & Memory 2*: 320-333.

Bolwig G., Del Vecchio M., Hannon G. & T. Tully (1995) Molecular cloning of *linotte* in *Drosophila*: a novel gene that functions in adults during associative learning. *Neuron 15*: 829-842.

Regulski M. & T. Tully (1995) Molecular and biochemical characterization of *dNOS*: a *Drosophila* Ca^{2+}/calmodulin-dependent nitric oxide synthase. *Proceedings of the National Academy of Science U.S.A. 92*: 9072-9076. **(114)**

Yin J.C.P., Wallach J.S., Zhou H., Klingensmith J., Du Y., Perrimon N., Tully T. & W.G. Quinn (1995) *Drosophila* CREB/CREM homolog encodes multiple isoforms including a PKA-responsive transcriptional activator and antagonist. *Molecular and Cellular Biology 15*: 5123-5130. **(31)**

Yin J.C.P., Del Vecchio M., Zhou H. & T. Tully (1995) CREB as a memory modulator: induced expression of an *hsp-dCREB2-a* activator isoform enhances the formation of long-term memory. *Cell 81*: 107-115. **(272)**

Tully T., Preat T., Boynton S.C. & M. Del Vecchio (1994) Genetic dissection of consolidated memory in *Drosophila melanogaster*. *Cell 79*: 35-47. **(237)**

Yin J.C.P., Wallach J.S., Del Vecchio M., Wilder E.L., Zhou H., Quinn W.G. & T. Tully (1994) Induction of a dominant-negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell 79*: 49-57. **(357)**

Tully T., Kruse L. & V. Cambiazo (1994) Memory through metamorphosis in normal and mutant *Drosophila melanogaster*. *Journal of Neuroscience 14*: 68-74. **(45)**

Tully T. & D. Gold (1993) Differential effects of *dunce* alleles on associative learning and memory. *Journal of Neurogenetics 9*: 55-71. **(30)**

Dura J.-M., Preat T. & T. Tully (1993) Identification of *linotte*, a new gene affecting learning and memory in *Drosophila melanogaster*. *Journal of Neurogenetics 9*: 1-14. **(65)**

Luo L., Tully T. & K. White (1992) Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for *Appl* gene. *Neuron 9*: 595-605. **(78)**

Boynton S. & T. Tully (1992) *latheo*, a new gene involved in associative learning and memory in *Drosophila melanogaster* identified from P element mutagenesis. *Genetics 131*: 655-672. **(70)**

Gailey D.A., Villella A. & T. Tully (1991) Reassessment of the effect of biological rhythm mutations on learning in *Drosophila melanogaster*. *Journal of Comparative Physiology A. 169*: 685-697. **(38)**

Publications (cont.)

McGuire T.R., Tully T. & A. Gelperin (1990) Conditioning odor-shock associations in the black blowfly *Phormia regina. Journal of Insect Behavior 3*: 49-59. **(3)**

McGuire T.R., McGuire R.K. & T. Tully (1989) A general program in PASCAL for biometrical genetic analysis of means. *Journal of Heredity 80*: 166. **(4)**

Hahn M.E., Hewitt J.K., Adams M. & T. Tully (1987) Genetic influences on ultrasonic vocalizations in young mice. *Behavior Genetics 17*: 155-166. **(20)**

McGuire T.R. & T. Tully (1987) Characterization of genes involved with classical conditioning that produce differences between bidirectionally selected strains of the blow fly *Phormia regina* . *Behavior Genetics 17*: 97-107. **(10)**

McGuire T.R. & T. Tully (1986) Food-search behavior and its relation to the central excitatory state (CES) in the genetic analysis of the blow fly *Phormia regina. Journal of Comparative Psychology 100*: 52-58. **(8)**

Reh T.A. & T. Tully (1986) Regulation of the number of tyrosine hydroxylase containing amacrine cells in the retina of *Rana pipiens* larvae. *Developmental Biology 114*: 463-469. **(117)**

Tully T. & J.P. Gergen (1986) Deletion mapping of the *Drosophila* memory mutant *amnesiac. Journal of Neurogenetics 3*: 33-47. **(23)**

Tully T. & W.G. Quinn (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster. Journal of Comparative Physiology 157*: 263-277. **(238)**

Tully T. & J. Hirsch (1983) Two new nonassociative components of the proboscis extension reflex in the blow fly, *Phormia regina* , which may affect measures of conditioning and of the central excitatory state (CES). *Behavioral Neuroscience 97*: 146-153. **(18)**

Tully T., Zawistowski, S. & J. Hirsch (1982) Behavior-genetic analysis of *Phormia regina* : III. A phenotypic correlation between the central excitatory state (CES) and conditioning remains in replicated F_2 generations of hybrid crosses. *Behavior Genetics 12*: 181-191. **(34)**

Tully T. & J. Hirsch (1982) Behavior-genetic analysis of *Phormia regina* : II. Detection of a single major-gene effect from behavioural variation for the central excitatory state (CES) using replicate hybrid crosses. *Animal Behaviour 30*: 1193-1202. **(30)**

Tully T. & J. Hirsch (1982) Behavior-genetic analysis of *Phormia regina* : I. Isolation of pure breeding lines for high and low levels of the central excitatory state (CES) from an unselected population. *Behavior Genetics 12*: 395-415. **(22)**

Invited Reviews (Citations)

Bolduc F. and T. Tully (2007) Molecular Mechanisms of Learning and Memory. (in press).

Wang Y., Dubnau J., Tully T. and Y Zhong (2007) Genetics in Learning and Memory. In *Neurobiology of Learning and Memory*, Kesner, R.P. and Martinez, J. (Eds), Elsevier. (in press).

Margulies C., Tully T. and J. Dubnau (2005) Deconstructing memory in *Drosophila*. *Current Biology 15*: R700-R713.

Tully T. (2003) Reply: The myth of a myth. *Current Biology 13*: R426.

Tully T., Bourtchouladze R., Scott R. and J. Tallman (2003) Targeting the CREB pathway for memory enhancers. *Nature Reviews Drug Discovery 2*:267-77. **(4)**

Tully T. (2003) Pavlov's flies. *Current Biology 13*: R117-119. **(1)**

Dubnau J., Chiang, A.-S. & T. Tully (2003) Neural substrates of memory: from synapse to system. *Journal of Neurobiology54*: 238-253. **(5)**

Tully T. (2002) Invertebrate Learning: neurogenetics of memory in *Drosophila*. In J.H. Byrne (Ed.) *Learning & Memory, Second Edition*. Macmillan Reference USA, Thomson Gale, New York, pp. 716.

Invited Reviews (cont.)

Fillit H.M., Butler R.N., O'Connell A.W., Albert M.S., Birren J.E., Cotman C.W., Greenough W.T., Gold P.E., Kramer A.F., Kuller L.H., Perls T.T., Sahagan B.G. and T. Tully T. (2002) Achieving and maintaining cognitive vitality with aging. *Mayo Clinic Proceedings*: 77:681-96. **(9)**

Dubnau J. & T. Tully (2001) Functional anatomy: from molecule to memory. *Current Biology 11*: R240-R243. **(9)**

Saitoe M. & T. Tully (2000) Making connections between synaptic and behavioral plasticity in Drosophila. In J. McEachern & C. Shaw (Eds.) *Toward a Theory of Neuroplasticity.* Psychology Press, New York, pp. 193-220.

Tully T. (1998) Toward a molecular biology of memory: the light's coming on! *Nature Neuroscience 1*: 543-545. **(16)**

Connolly J.B. & T. Tully (1998) Integrins: Adhering to a role for mushroom bodies in olfactory memory. *Current Biology 8*: R386-389. **(9)**

Dubnau J. & T. Tully (1998) Gene discovery in *Drosophila*: new insights for learning and memory. *Annual Review Neuroscience* 21: 407-444. **(134)**

Tully T. (1997) Regulation of gene expression and its role in long-term memory and synaptic plasticity. *Proceedings of the National Academy of Science USA 94*: 4239-4241. **(27)**

Silva A.J., Simpson E.M., Takahashi J.S., Lipp H.-P., Nakanishi S., Wehner J.M., Giese K.P., Tully T., Abel T., Chapman P.F., Fox K., Grant S., Itohara S., Lathe R., Mayford M., McNamara J.O., Morris R.J., Picciotto M., Roder J., Shin H.-S., Slesinger P.A., Storm D.R., Stryker M.P., Tonegawa S., Wang Y., and D.P. Wolfer (1997) Mutant Mice and Neuroscience: Recommendations Concerning Genetic Background . *Neuron 19*: 755-759. **(180)**

Tully T., Bolwig G., Christensen J., Connolly J., DelVecchio M., DeZazzo J., Dubnau J., Pinto S., Regulski M., Svedberg B. & K. Velinzon (1996) A return to genetic dissection of memory in *Drosophila. Cold Spring Harbor Symposium on Quantitative Biology 61*: 207-218. **(14)**

Tully T. (1996) Discovery of genes involved with learning and memory: an experimental synthesis of Hirschian and Benzerian perspectives. *Proceedings of the National Academy of Science USA 93*: 13460-13467. **(47)**

Yin J. & T. Tully (1996) CREB and the formation of long-term memory. *Current Opinions in Neurobiology 6*: 264-268. **(109)**

Connolly J.B. & T. Tully (1996) You must remember this: finding the master switch for long-term memory. *The Sciences 36*: 37-42.

DeZazzo J & T. Tully (1995) Dissection of memory formation: from behavioral pharmacology to molecular genetics. *Trends in Neuroscience 18*: 212-218. **(137)**

Tully T. (1994) Gene disruption of learning and memory: a structure-function conundrum? *Seminars in Neuroscience 6*: 59-66. **(6)**

Tully T. (1991) *Drosophila*'s role in identifying the building blocks of associative learning and memory. In R.G. Lister and H.J. Weingartner (Eds.) *Perspectives in Cognitive Neuroscience.* Oxford, Oxford University Press, pp. 46-64. **(8)**

Tully T. (1991) Physiology of mutations affecting learning and memory in *Drosophila* - the missing link between gene product and behavior. *Trends in Neuroscience 14*: 163-164. **(16)**

Tully T. (1991) Genetic dissection of learning and memory in *Drosophila melanogaster* . In J. Madden (Ed.) *Neurobiology of Learning, Emotion and Affect.* Raven Press, New York, pp. 29-66. **(26)**

Tully T., Boynton S., Brandes C., Dura J.M., Mihalek R., Preat T. & Villella A. (1990) Genetic dissection of memory formation in *Drosophila melanogaster. Cold Spring Harbor Symposium on Quantitative Biology 55*: 203-211. **(47)**

Boynton S. & T. Tully (1990) Induction of a long-term memory in *Drosophila* is not affected by protein synthesis inhibitors. In E. Lindenlaub (Ed.) *Symposia Medica Hoechst 23: The Biology of Memory.* Verlag, Berlin, pp. 91-102.

Invited Reviews (cont.)

Tully T. (1988) What is the most expedient approach to understanding the proximate causes of aging? *Experimental Gerontology 23*: 269-270.

Tully T. (1988) On the road to a better understanding of learning and memory in *Drosophila melanogaster.* In G. Hertting & H.-Ch. Spatz (Eds.) *Modulation of Synaptic Transmission and Plasticity in Nervous Systems*. NATO ASI Series, Springer-Verlag, Berlin, pp. 401-417. **(2)**

Tully T. (1987) *Drosophila* learning and memory revisited. *Trends in Neuroscience 10*: 330-335.

Tully T. (1986) Measuring learning in individual flies is not necessary to study the effects of single-gene mutations in *Drosophila. Behavior Genetics 16*: 449-455. **(9)**

Tully T (1984) *Drosophila* Learning: Behavior and Biochemistry. *Behavior Genetics 14:* 527-557. **(51)**

Aceves-Pina E.O., Booker R., Duerr J.S., Livingstone M.S., Quinn W.G., Smith R.F., Sziber P.P., Tempel B.L. & T. Tully (1983) Learning and memory in *Drosophila*, studied with mutants. *Cold Spring Harbor Symposium on Quantitative Biology 48*: 831-840. **(8)**

Hirsch J. & T.P. Tully (1982) The challenge is unmet. [Continuing Commentary on A.R. Jensen, 1980].*The Behavioral and Brain Sciences 5*: 324-326.

Hirsch J., Beeman M. & T.P. Tully (1980) Compensatory education has succeeded. [Review of A.R. Jensen (1980) *Bias in Mental Testing*]. *The Behavioral and Brain Sciences 3*: 368-371.

EXHIBIT

Motor impairments represent the most common form of disability resulting from stroke. Depending on the severity of the impairments, motor rehabilitation can result in significant improvements in motor function over time. The efficacy of the phosphodiesterase inhibitor HT0712 ((3S, 5S)-5-(3-cyclopentyloxy-4-methoxy-phenyl)-3-(3-methyl-benzyl)-piperidin-2-one; also known as IPL 455,903)) in promoting rehabilitation-dependent motor recovery and enhancing functional restoration within the motor cortex following cortical ischemia was examined.

Materials and Methods

Subjects: Forty-two adult (90 days) male Long-Evans hooded rats (350-420g) were group housed (2 animals/cage) in standard laboratory cages on a 12:12 hour light dark cycle throughout the experiment.

Reach Training: Over the course of several days, all animals were placed on a restricted diet until they reached 90% of their original body weight. A brief period of pretraining was then given to familiarize the rats with the reaching task. This involved placing the animals into test cages (10 X 18 X 10 cm) with floors constructed of 2 mm bars, nine mm apart edge to edge. A four cm wide and 5 cm deep tray filled with food pellets (45 mg; Bioserv) was mounted on the front of the cage. The rats were required to reach outside the cage and retrieve pellets from the tray. All rats remained in pretraining until they had successfully retrieved 10 pellets (approximately 1 hour/day for 2 days). After pretraining, the rats were placed into a Plexiglas cage (11 cm X 40 cm X 40 cm) with a 1 cm slot located at the front of the cage. Animals were trained for 20 minutes each day to reach through the slot and retrieve food pellets from a table outside the cage. Rats were permitted to use either limb and the preferred limb was noted for each animal. Each session was videotaped and later used to assess reaching performance. A successful reach was scored when the animal grasped the food pellet, brought it into the cage and to its mouth without dropping the pellet. The percentage of successful reaches [(# successful retrievals/the total # of reaches) x 100] was then calculated. Animals were trained for

approximately 2 weeks on this task to establish a baseline measure of motor performance. Baseline was defined as the average accuracy across the three final days of training. Post stroke performance was expressed as a percentage of the baseline performance.

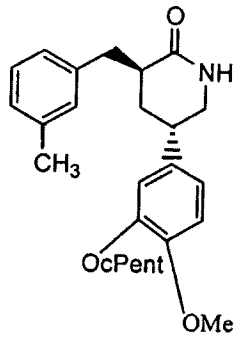
Electrophysiological Mapping: Within 2 days of the final training session, standard intracortical microstimulation (ICMS) techniques were used to generate detailed maps of forelimb regions of the motor cortex contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). Animals received low levels of isofluorane (0.15%) and supplemental doses of ketamine (20 mg/kg i.p.) as needed. Under sterile conditions, a craniotomy was performed over the motor cortex contralateral to the trained paw of each animal. To prevent edema, a small puncture was made in the cisterna magna prior to removing the skull and dura. The exposed cortex was then covered in warm saline (37°C). A digital image of the cortical surface was taken and a 375 □m grid was superimposed onto the image. A glass microelectrode (controlled by a hydraulic microdrive) was used to make systematic penetrations across the cortex using the cortical surface image and grid as a guide. At each penetration site, the electrode was lowered to approximately 1550 μm (corresponding to cortical layer V). Stimulation consisted of 13, 200 μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position with the limb consistently supported. Sites where no movement was detected at ≤ 60 μA were recorded as unresponsive. Forelimb movements were classified as either distal (wrist/digit) or proximal (elbow/shoulder) and representational maps were generated from the pattern of electrode penetrations. The caudal forelimb area (CFA) was defined by a medial boundary of vibrissa representations, a lateral and caudal boundary of non response sites and a rostral boundary of head and neck representations. An image analysis program (CANVAS v. 3.5) was used to calculate the area extent of the caudal forelimb area (CFA).

Focal Infarction: Focal ischemic infarcts were created within caudal forelimb area via bipolar electrocoagulation of the surface vasculature. The infarct targeted primarily the distal forelimb representations but in some cases included small regions of proximal representations. The coagulated vessels included fine arterial and venous capillaries as well as larger vessels but specifically avoided any bypassing arteries supplying other cortical areas. Coagulation was

continued until all vessels within the targeted area were no longer visible and the tissue appeared white.

Motor Rehabilitation: Within three days of the initial mapping and infarction procedure, all animals were placed into a motor rehabilitation program that consisted of being trained daily for 15 minutes on the skilled reaching task described above for 10 days. Animals were also randomly assigned to one of five doses of HT0712: Vehicle (n=8), 0.10 mg/kg (n=7), 0.15mg/kg (n=8), 0.30 mg/kg (n=9), and 0.10 mg/kg given twice per day. All animals received injections 20 minutes prior to the daily training session with the exception of one group of 0.10 mg/kg animals that received a second injection 3 hours after training. The sessions were video taped and reaching accuracy was assessed as described above.

HT0712 has the following formula:



wherein "Me" means "methyl" and "cPent" means "cyclopentyl". HT0712 can be prepared using the methodology provided in U.S. Patent No. 6,458,829B1.

Assessing Cortical Dysfunction: Within one day of the final training session, ICMS was again used to generate a second map of the caudal forelimb area (CFA) contralateral to the trained forelimb. Prior to surgery animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.), receiving xylazine (0.02 mg/kg i.m.) and ketamine (20 mg/kg i.p.) as needed. Further, animals were placed on isoflurane (.15%, 1.5% O_2) when needed. The dental polymer, gel film and gel foam were removed and the exposed cortex covered in warm silicon oil. Mapping procedures were identical to those used in the initial mapping.

Results

Skilled Reaching: A repeated measures Analysis of variance (ANOVA) with DAY as a within subjects factor and CONDITION as a between subject factor revealed a significant DAY X CONDITION interaction [$F(9,36) = 1.72$; $p<0.05$) on reaching performance. Subsequent multiple comparisons (Fishers PLSD; $p <0.05$) showed the 0.15 mg/kg and 0.10 twice per day HT0712 injected animals to have a significantly higher reaching accuracy than all other groups during the later stages of training. The 0.10 mg/kg animals had significantly better reaching accuracies than the Vehicle and 0.30 mg/kg HT0712 animals.

Map Area: An analysis of variance with CONDITION as a between subject factor revealed a no significant effect of CONDITION on Map 1 area [$F (4,36) = 1.1$ $p>0.05$]. A significant main effect of CONDITION on Map 2 [$F(4,36) = 6.5$; $p<0.05$]. Subsequent multiple comparisons (*Fishers PLSD; $p<0.05$) showed the 0.10 mg/kg had significantly 0.10 mg/kg twice/day and the 0.15 mg/kg all had significantly larger Map 2 than the 0.30 mg/kg and Vehicle injected animals.

Discussion

Functional impairments following brain injury are due to both the loss of tissue within the damaged area and concomitant dysfunction within other brain area. The results of the present study show that recovery, which is associated with a reinstatement and reorganization of function within residual tissue, can be upregulated via the inhibition of phosphodiesterase in combination with rehabilitative training. Further, the expansion of movement representations within peri-infarct areas was accompanied by enhanced motor recovery. The increase in peri-infarct motor map area represents the restoration of cortical circuitry that is augmented through the upregulation of cAMP. The results of the present study demonstrate a dose dependent increase in motor recovery and enhanced functional restoration within motor cortex. With the exception of the animals receiving 0.30 mg/kg, all animals receiving HT0712 in combination with motor

rehabilitation had significantly larger motor maps and better post-stroke reaching performance than vehicle injected controls.

The results indicate that HT0712 contributes to recovery of motor function by augmenting the restoration of cortical function that occurs during rehabilitation. Specifically, HT0712 may act to facilitate synaptic strengthening and the reinstatement of the cortical circuitry required to support both the motor maps and skilled motor behavior.